Supporting Information

Assembly and solution phase properties of Ag^+–mediated adenine-thymine DNA duplexes

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1 Methods

Formation of Ag^+-mediated A:T duplex. ssDNA (purchased from Integrated DNA Technologies with standard desalting) was further desalted using NAP-5 columns packed with Sephadex™ G-25 DNA grade gel. Concentration of desalted DNA stock was determined using optical absorbance values at 260 nm measured on a NanoDrop microvolume UV-Vis spectrophotometer (ε_{260} for dA_{20} and dT_{20} are 243400 and 162600 L mol^{-1} cm^{-1}, respectively). Samples were prepared by combining DNA and $AgNO_3$ in 10 or 50 mM ammonium acetate (NH_4OAc) , pH 7 and mixed by vortexing. Samples were annealed by heating at 90 °C for 10 minutes with an Eppendorf Nexxus Gradient MasterCycler thermal cycler before further characterization.

CD spectroscopy. CD spectra were collected on a Chirascan V100 spectrometer using 0.5 mm pathlength quartz cuvettes. Measurements were performed in triplicate over a range of 210 nm to 350 nm with 1 nm bandwidth, 1 nm scanning intervals, and 0.75 s integration time. Triplicate spectra were averaged and then smoothed by averaging two neighboring points on either side of the data point used in the analysis. Data collected from the instrument was measured in millidegrees (mdeg). Values in mdeg were converted to molar ellipticity ($deg \bullet cm^2 \bullet dmol^{-1}$) to correct for concentration and compare spectra from different samples. The mdeg units were not converted for temperature studies, as the concentration at higher temperatures is uncertain due to possible evaporation of solution.

The CD instrument is equipped with a single sample holder attachment with a Peltier module for controlled temperature experiments. Room temperature measurements were performed at 20 °C. To obtain the melting temperature (T_m) of the duplex, pre-annealed solutions were heated from 20 to 90 °C with a heating rate of 1 ºC min^{-1}. T_m is determined by plotting the CD intensity at a selected characteristic wavelength as a function of temperature, and the resulting curve is fitted

to a sigmoid function by least squares fitting (Wavemetrics, Inc., Igor Pro software) to determine T_m, which is derived from the inflection point of the sigmoidal curve.

Stopped flow CD. DNA sample solution was rapidly mixed with $AgNO_3$ stock solution at a volume ratio of 1:1. Final concentration in the 10 mm pathlength observation cell was 20 μM DNA and 0.2 mM $AgNO_3$ (0.5 Ag^+/base); 20 μM DNA was used to ensure sufficient light transmission through the longer 10 mm pathlength of the stopped flow accessory, which is in line with the same lamp and detector used for temperature resolved CD experiments. Bandwidth was set to 4 nm, and time points were collected every 2 ms for a total duration of 1 s. The experiment was performed in triplicate, and averaged data was then fitted to a single exponential function $y(t) = y_0 + Ae^{-t/\tau}$ (using Igor Pro), yielding time constant, $\tau = 57.7 \pm 0.7$ ms.

Isothermal titration calorimetry (ITC). ITC experiments were performed using a TA instruments NanoITC. The sample cell containing dA_{20}:dT_{20} DNA solution in 50 mM NH_4OAc (pH 7) was held at a constant temperature of 25 ºC, and $AgNO_3$ was injected into the cell. A total volume of 10 μL of $AgNO_3$ was injected in a series of 25 injections into 950 μL of DNA solution. To account for background effects, heats of dilution were measured by adding $AgNO_3$ to the 50 mM NH_4OAc and adding 50 mM NH_4OAc to the DNA solution. Thermodynamic parameters like change in enthalpy (ΔH), entropy (ΔS), Gibbs free energy (ΔG), association constant (K_a), and reaction stoichiometry (n) were derived from the data using the independent model. The initial binding curve could be deconvoluted to form two sigmoidal curves that represent two binding events. Different concentrations of $AgNO_3$ were injected to ensure reproducibility as well as to isolate the second binding event.

FRET studies. HPLC-purified ssDNA strands labelled with Cy3 or Cy5 were purchased from Integrated DNA Technologies. Dyes are attached either at the 5′-end or the 3′-end. The dA_{20} strand includes two T bases between the A_{20} sequence and the dye molecule as spacers to allow for free dye rotation. Cy3 was excited at 520 nm using an HPX-2000 Xenon lamp (Ocean Insight) coupled with a Monoscan 2000 monochromator (Ocean Optics). Absorbance and emission spectra were collected using an Ocean Optics QE65000 spectrometer.

Cryo-transmission electron microscopy. DNA sequences used for cryo-EM are dA_{40} and dT_{40}; longer sequences were chosen to make image acquisition easier with larger samples. Length was limited to 40 bases by the manufacturer. DNA samples were prepared at a total DNA concentration of 100 μM. R2/2 Quantifoil grids with copper 400 mesh were made hydrophilic by glow discharge for 30 s using PIE Scientific Tergeo Sample Cleaner. Vitrification was performed using a FEI Vitrobot™ Mark III in a humidity-controlled room at 20% humidity during sample preparation. Ultrapure water was filled in the humidifier of the Vitrobot, and chamber humidity was set to 95% and temperature at 22 ºC. 5 μL droplets were pipetted onto the grid and then blotted with two filter papers with blot force of 11 and blot time of 2.5 s. Grids were plunged into liquid ethane at *ca.* –180 ºC. Samples were then transferred to a grid clipping station before loading onto the Glacios cryo-TEM autoloader. Cryo-TEM performed on Thermo Scientific Glacios Cryo-TEM (X-FEG 200 kV electron source) and data collected using Thermo Scientific Ceta camera. Imaging was performed using a parallel beam (nanoprobe mode) with a dose rate of ~2800 – 3100 $e^{-}Å^{-2}s^{-1}$ for each image.

2 CD spectra of dA_{20} and dT_{20} with Ag^+

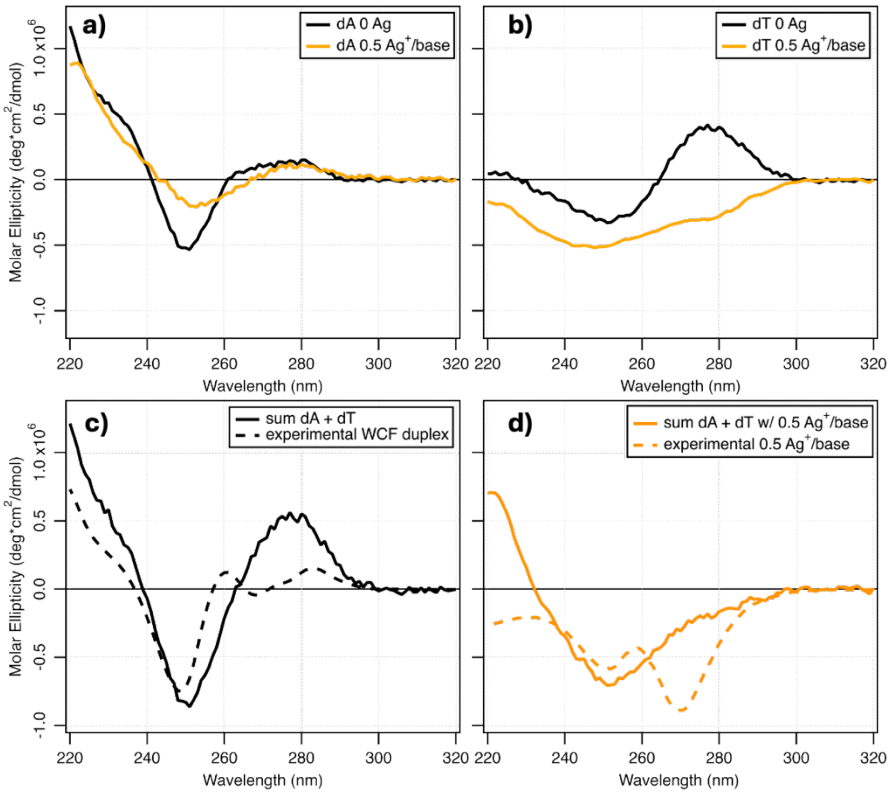


Figure S1: CD spectra **a)** dA_{20} and **b)** dT_{20} with (**orange**) and without (**black**) Ag^+. **c)** Comparing the sum of dA and dT without Ag^+ (black solid) to experimental CD data of the WCF duplex (black dashed). **d)** Comparing sum of dA 0.5 [Ag^+]/[base] + dT 0.5 [Ag^+]/[base] (orange solid) with experimental data of dA–[Ag^+]–dT (orange dashed) formed with 0.5 [Ag^+]/[base]. *Conditions:* 15 µM DNA, 10 mM NH_4OAc, pH 7, 20 °C.

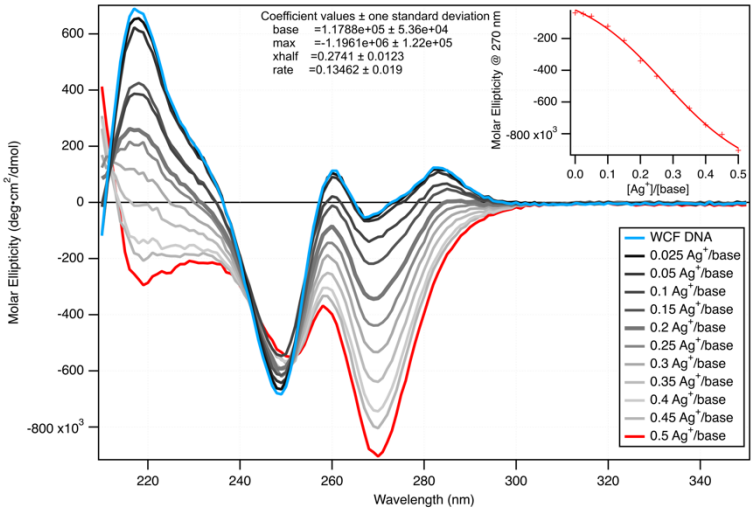


Figure S2: Changes in CD spectra of dA:dT WCF duplex upon addition of smaller increments of [Ag^+]/[base] up to 0.5 [Ag^+]/[base], forming dA–Ag^+–dT duplex. Inset tracks the change of molar ellipticity with [Ag^+]/[base] increase. *Conditions:* 40 µM DNA, 50 mM NH_4OAc, pH 7, annealed to 90 ºC measured at 20 ºC.

3 Isothermal Titration Calorimetry

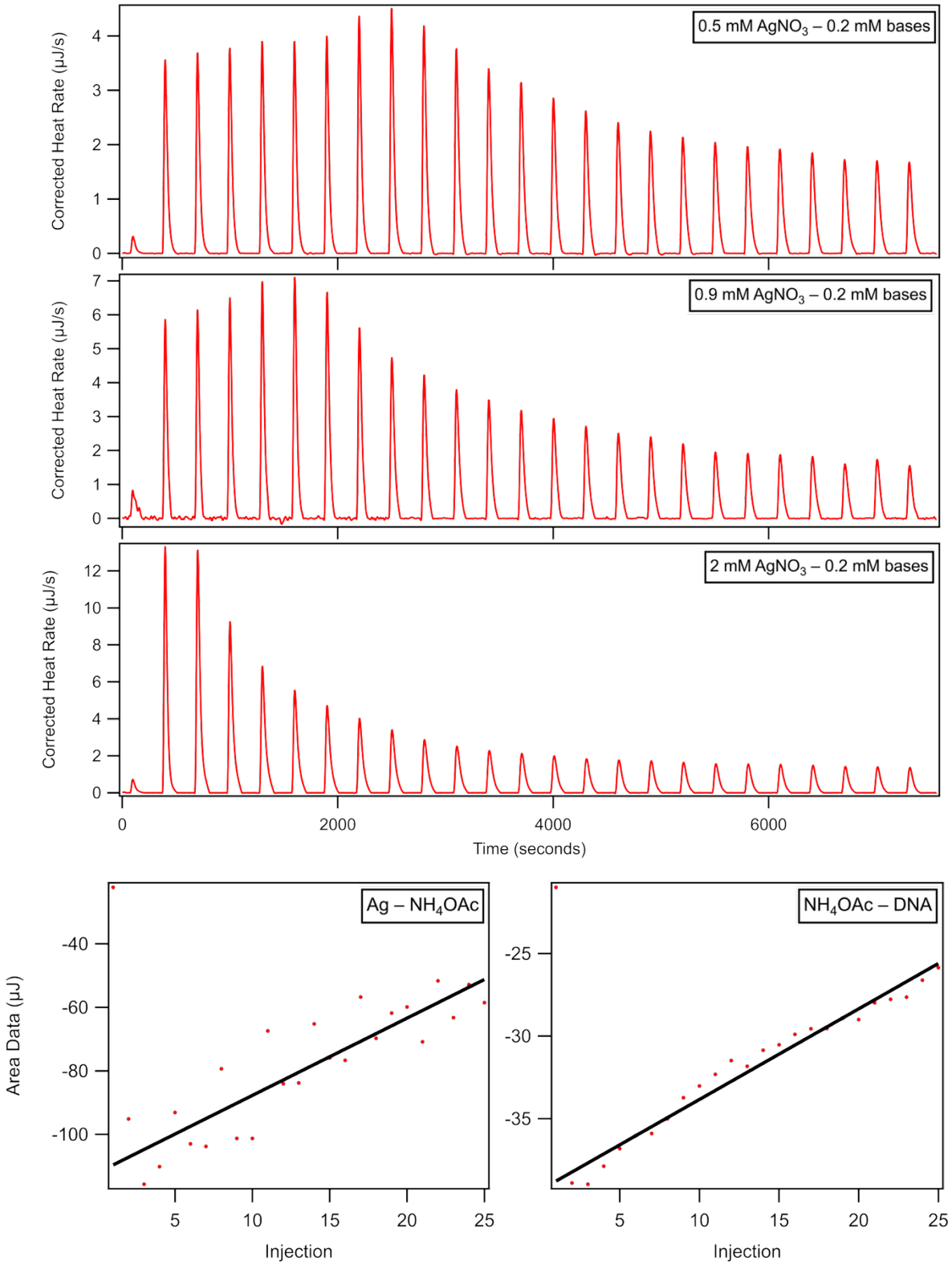
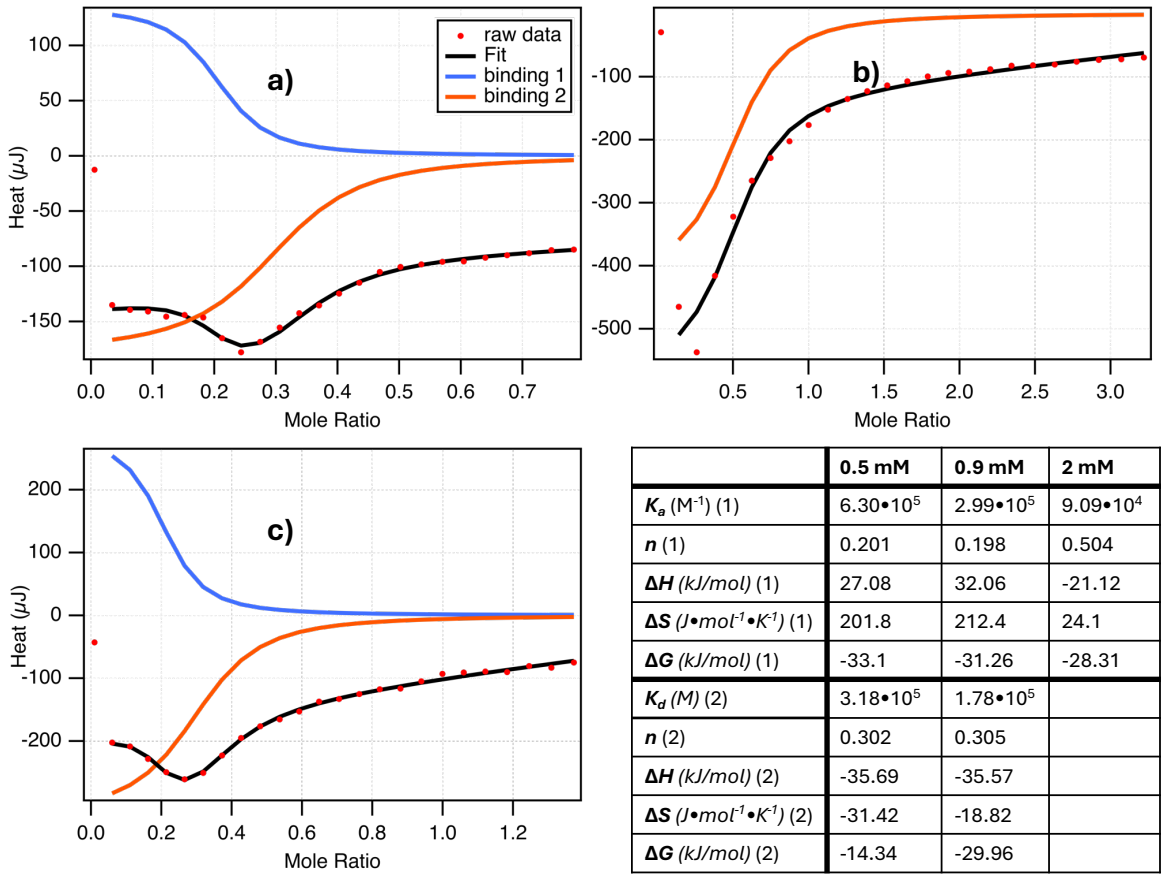


Figure S3: ITC thermograms: **a)** raw heat rate graphs with varying ratios of $AgNO_3$ injected into 0.2 mM DNA base, **b)** heat of dilution into 50 mM NH_4OAc (pH 7) for correcting raw data.



	0.5 mM	0.9 mM	2 mM
K_a (M^{-1}) (1)	$6.30 \cdot 10^5$	$2.99 \cdot 10^5$	$9.09 \cdot 10^4$
n (1)	0.201	0.198	0.504
Δ*H* *(kJ/mol)* (1)	27.08	32.06	-21.12
Δ*S* *($J \cdot mol^{-1} \cdot K^{-1}$)* (1)	201.8	212.4	24.1
Δ*G* *(kJ/mol)* (1)	-33.1	-31.26	-28.31
K_d *(M)* (2)	$3.18 \cdot 10^5$	$1.78 \cdot 10^5$	
n (2)	0.302	0.305	
Δ*H* *(kJ/mol)* (2)	-35.69	-35.57	
Δ*S* *($J \cdot mol^{-1} \cdot K^{-1}$)* (2)	-31.42	-18.82	
Δ*G* *(kJ/mol)* (2)	-14.34	-29.96	

Figure S4: ITC data with deconvolution of the two binding events to derive the thermodynamic parameters. **a)** 0.5 mM, **b)** 2 mM, and **c)** 0.9 mM $AgNO_3$ injected into 2 mM DNA base to demonstrate reproducible binding behavior. The table shows the derived thermodynamic parameters for each $AgNO_3$ concentration.

4 CD measurements of dye-labelled duplexes

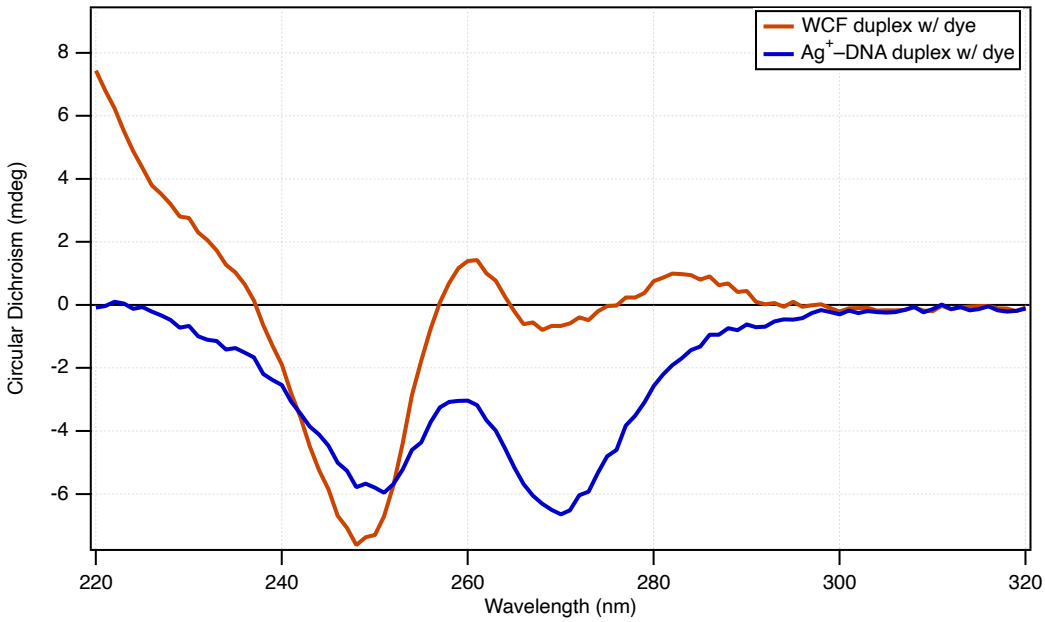


Figure S5: CD spectra of **5′-Cy5-dT$_{20}$-3′** and **5′-Cy3-dA$_{20}$T$_2$-3′** strands assembled as WCF duplex (**red**) and with 0.5 [Ag$^+$]/[base] (**blue**). *Conditions:* 2.5 µM DNA, 50 mM NH_4OAc (pH 7), annealed to 90 ºC and cooled to room temperature. The signal-to-noise ratio is lower than in Figure 1 due to lower DNA concentrations to conserve dye-labelled strands.

5 Cryo-TEM images

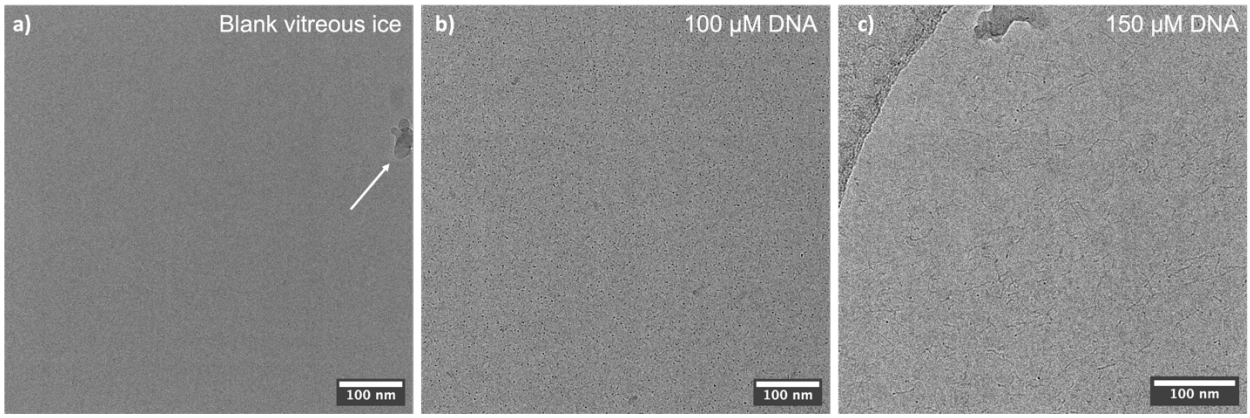


Figure S6: Cryo-TEM micrographs of **a)** empty vitreous ice with no DNA (image selected to provide example of ethane contamination, indicated by white arrow). **b)** WCF **dA$_{40}$:dT$_{40}$** duplexes at 100 µM and **c)** 150 µM DNA, collected at 95k times magnification.

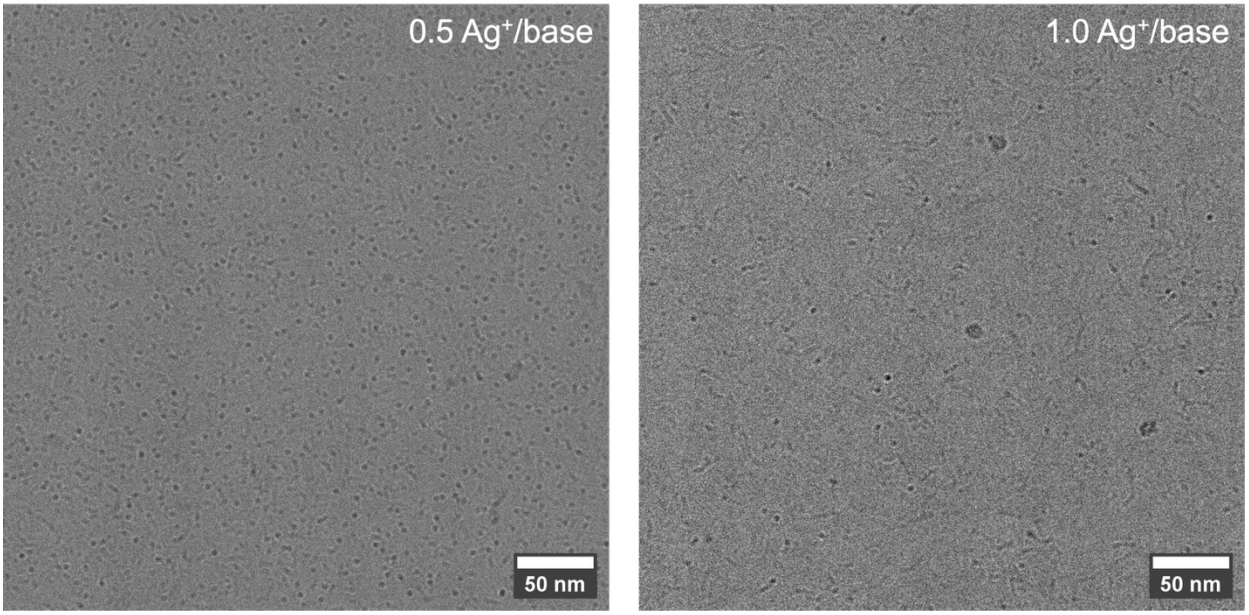


Figure S7: Cryo-TEM micrographs of **(dA_{40})–$[Ag^+]_M$–(dT_{40})** duplexes at 0.5 (left) and 1.0 (right) $[Ag^+]$/base stoichiometries, collected at 150k times magnification.

6 Temperature-dependent CD spectroscopy

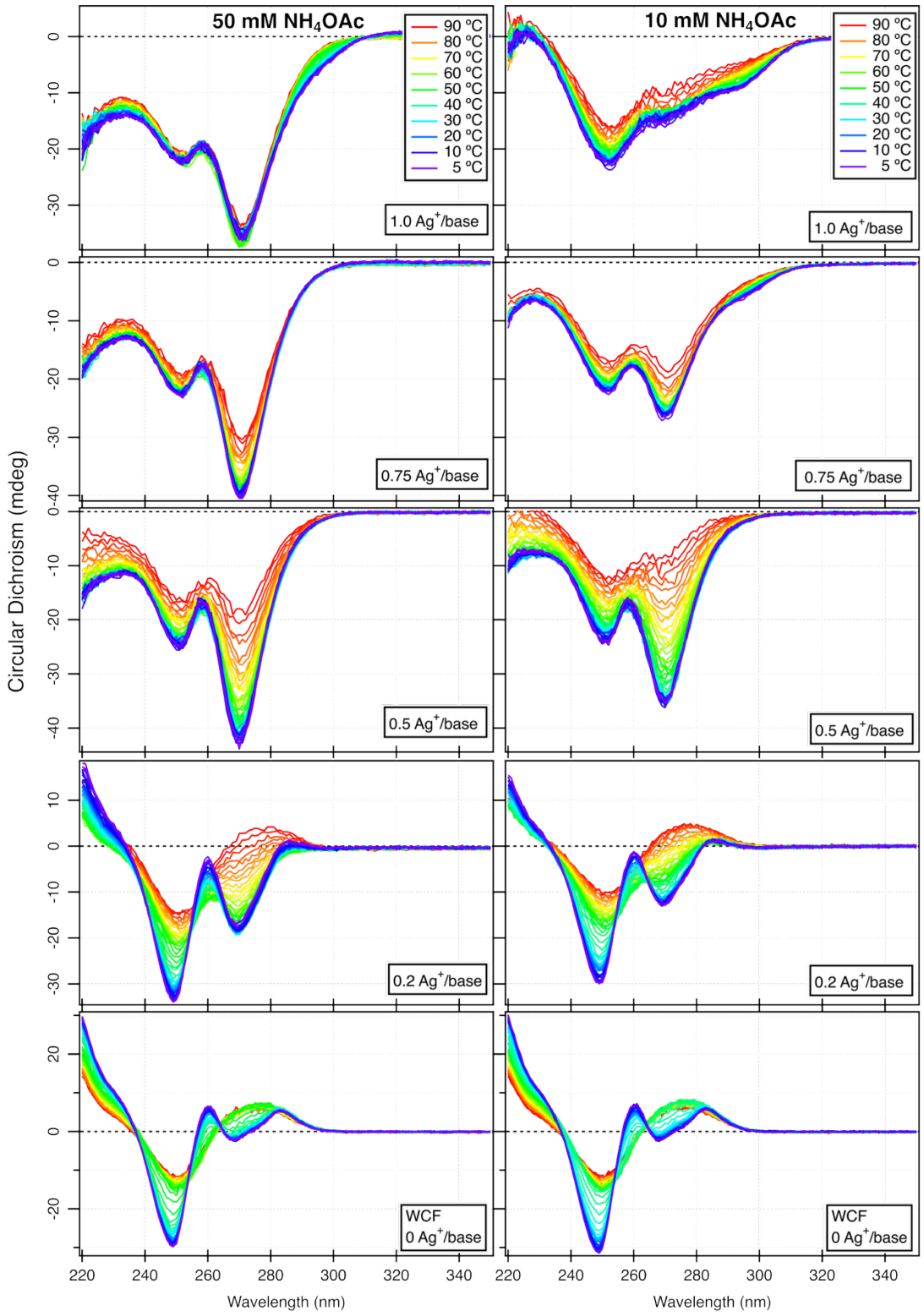


Figure S8: Comparison of temperature-dependent CD spectra of **WCF dA:dT** duplex with **dA–[Ag⁺]–dT** duplex assembled with increasing [Ag⁺/[base] concentration in 50 mM (*left column*) and 10 mM NH_4OAc (*right column)*. *Conditions:* 40 μM DNA in NH_4OAc (pH 7), annealed to 90 ºC before experiment.

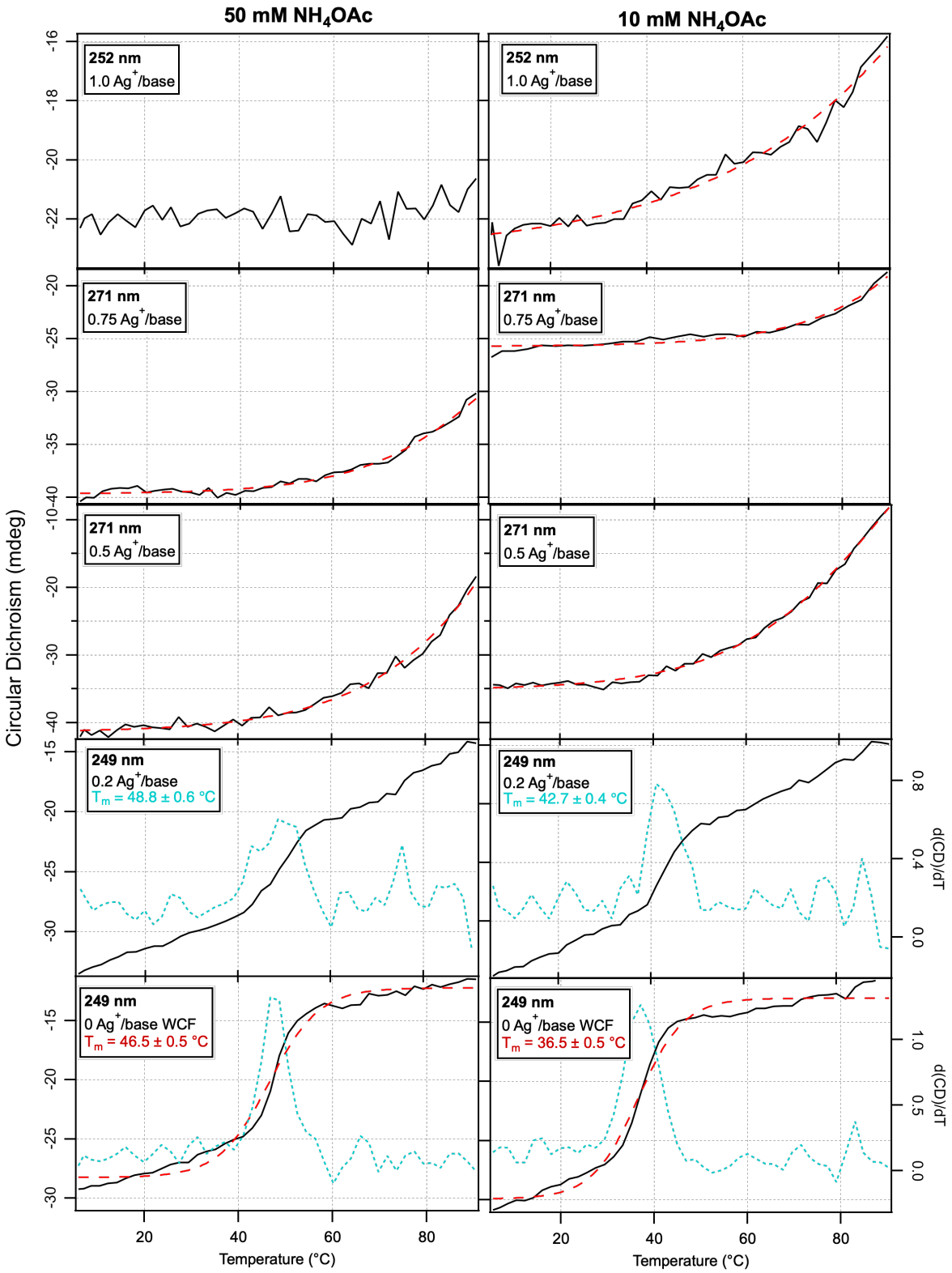


Figure S9: CD vs. temperature (black) at varying $AgNO_3$ and NH_4OAc (pH 7) concentrations. Sigmoidal fits (**red**) are used to determine T_m for WCF samples. T_m for 0.2 Ag^+/base samples is determined by Gaussian fits to 1st derivative of CD vs. temperature (**teal**) due to the linear increase of CD before and after the observed melting transition.